INFLUENCE OF AGE AND GENDER ON ETHANOL METABOLISM IN RAT LIVER

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Studies on the extent to which aging alters the metabolism and disposition of ethanol have yielded conflicting results, with elimination rates and metabolic rates reportedly decreasing, increasing or remaining unchanged in old age. Our studies in 4-, 14- and 25-month-old Fischer 344 rats indicated that liver alcohol dehydrogenase activity (ADH) was lower in mature male rats than in females. The activity in males increased with age, and the sex-dependent difference was diminished in old age. Ethanol elimination rates in aging male F344 rats *in vivo* reflect the age-related changes in ADH; however, these alterations may be absent in strains that do not display a gender difference in ADH. We also measured rates of microsomal ethanol oxidation (MEOS) in livers of F344 rats as a function of age and sex. MEOS was greater in liver microsomes of mature males than in those of females. The activity in males declined in old age, but the activity in females was unchanged by aging. The results indicate that age-associated changes in ethanol metabolism are subject to gender differences. This work was supported by NIH Grant No. AG04984 and a contract from the Oklahoma Center for the Advancement of Science and Technology.

COMPARISON OF THE METABOLISM OF EFLUMAST (RP42068) IN THE ISOLATED PERFUSED RAT LIVER AND HEPATOCYTE SUSPENSIONS

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The metabolism of eflumast (RP42068), an anti-allergic agent, was studied in the isolated perfused rat liver and hepatocyte suspensions. The method of Brauer et al. (1951) was employed for isolated liver perfusion and that of Paine and Hockin (1982) and Fry (1981) for obtaining viable suspensions of hepatocytes. [^{14}C]RP42068 was added to the perfusate (Kreb's buffer/blood; 2:1) of the liver perfusions and re-circulated for 2 hours and to the hepatocyte suspensions for incubating at 37°C for 3 hours. Samples were removed from both at intervals during the perfusion/incubation period. After 2 hours perfusion, the majority of the radioactivity was present in the plasma perfusate (69-76%) with 3-6% in the bile and about 5% remaining in the liver. Using thin layer chromatography, perfusion bile was shown to contain 3 radiolabelled products (metabolites) the major one of which was identified as an N-glucuronide. Perfusion plasma contained an additional 4 metabolites, none of which was an N-glucuronide. Hepatocytes obtained by the method of Fry (1981) - a collagenase digestion of liver slices - gave the best metabolite profile (a minimum of 5 metabolites, one of which was identified as an N-glucuronide). Four of the metabolites produced in the incubation were also produced in the perfusion. Though not providing a complete metabolite profile, most of the major radiolabelled products produced during the liver perfusions were also produced during the incubation of hepatocyte suspensions.

Brauer, R.W., R.L. Pessotti and P. Pizzolato (1951) Proc. Soc. Exp. Biol. Med. 78 174

Paine, A.J. and Hockin, L.J. (1982) Toxicology 25 41

Fry, J.R. (1981) Methods Enzymol. 77 130

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CLONING AND EXPRESSION OF cDNAs FOR FIVE MEMBERS OF THE HUMAN CYTOCHROME P4502C SUBFAMILY AND CORRELATION OF 2C18 WITH (S)-MEPHENYTOIN HYDROXYLASE PHENOTYPE.

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The cytochrome P450 mediated metabolism of S-mephenytoin is a well-known genetic polymorphism in humans. This P450 belongs to the 2C subfamily, but the specific isozyme responsible for this polymorphism has not been identified unequivocally. In the present study, we isolated 83 essentially full length 2C clones from a human liver cDNA library. cDNAs representing 5 members of this subfamily are described including three new members of the human P4502C subfamily. Full length clones were isolated for 2C8 and two allelic variants of 2C9 (clones 25 and 65). Clone 254c is a partial cDNA representing a new P450 (2C17). Clones 29c and 6b represent full-length allelic variants of a new P450 (2C18), differing by one amino acid. Clone 11a represents a full length clone for a third new P450 (2C19). Hybridization analysis and partial sequencing indicated that 50 of the 83 clones isolated from this library were allelic variants of 2C9, 29 were 2C8, 3 were allelic variants of 2C18 and 1 was 2C19. 2C18 and 2C19 are more homologous to 2C17 than to 2C8 or 2C9. cDNAs for 2C9, 2C18 and 2C19 were expressed in COS-1 cells. Only COS-1 cells transfected with 2C18 metabolized racemic mephenytoin at a rate higher than background. Moreover, there was a strong correlation between the rate of S-mephenytoin 4-hydroxylation in liver microsomes of humans (rates varied 18-fold) and hepatic 2C18 mRNA content. These studies indicate that P4502C18 appears to be a major cytochrome responsible for (S)-mephenytoin 4-hydroxylation in humans.

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METABOLISM AND BIOREDUCTIVE ACTIVATION OF MITOMYCIN C BY DT-DIAPHORASE.

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The role of DT-Diaphorase (DTD) in the bioreductive activation of the antitumor quinone Mitomycin C (MC) was investigated. Metabolism of MC by purified rat hepatic DTD was found to be pH-dependent. No metabolism of MC could be detected at pH 7.8, but metabolism increased as the pH was decreased to 5.8. One major metabolite was isolated during metabolism of MC by DTD at acidic pH and this was characterized as 2,7-diaminomitosene. Metabolism of MC by DTD at acidic pH could be inhibited by dicumarol, an inhibitor of DTD or by prior boiling of enzyme and was NADH or NAD(P)H-dependent. No oxygen uptake could be detected during incubation of MC with DTD and NADH at either pH 5.8 or 7.8. The mechanism underlying pH-dependent metabolism of MC by DTD was then examined. Incubation of MC with NADH and DTD at pH 7.8 led to effective inhibition of DTD, whereas no inhibition was observed at pH 5.8. The influence of pH on MC-induced inhibition of DTD probably reflects the ambivalent nature of the quinone methide derived from MC after reduction. At pH 7.8, the quinone methide can act as an electrophile resulting in alkylation of the enzyme and loss of catalytic activity. At pH 5.8, the quinone methide can be protonated leading to 2,7-diaminomitosene formation and preservation of enzyme activity. That metabolism of MC by DTD at acidic pH represented a true activation step was confirmed using DNA damage and glutathione conjugate formation as indicators of bioactivation. MC-induced DNA binding, glutathione conjugate formation and DNA cross-linking during metabolism by DTD could not be detected at pH 7.8, but became increasingly evident as the pH was lowered to 5.8 and could be inhibited by dicumarol. These data show that DTD is capable of bioreductive activation of MC and that this process is pH-dependent. Supported by NCI RO1 CA51210).

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EFFECT OF HUMAN DIETARY MACROCOMPONENTS ON HEPATIC ACTIVATION OF FOOD MUTAGENS AND PRESENCE OF DNA ADDUCTS IN HUMAN-FLORA-ASSOCIATED (HFA) RATS.

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The aim of this research was to study the effect of diet on potential initiation steps in carcinogenesis. Four groups of 5 male germfree F344 rats were dosed orally with a human faecal suspension. At 7 weeks, the rats were fed one of the following isocaloric human diets: low fat (LF), low fibre (LFb), low beef (LB); high F, LFb, LB; LF, high Fb, LB; LF, LFb, high B. After 4 weeks, the rats were killed and livers removed. A portion was used for ^{32}P post-labelling assay to estimate the background DNA adducts (relative adduct levels; RAL) and the remainder used for preparation of hepatic S9. The S9 fractions were incubated with 2-amino-3-methyl-3H-imidazo[4,5-f]quinoline (IQ) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PHIP) in the Salmonella mutagenicity test, using S. typhimurium TA98 as indicator strain.

Activation of PHIP by S9 was greatest with the high fat diet giving 979±150 revertants compared with 582±257 revertants for rats fed the LF, LFb, LB diet. Activation of IQ was more than doubled with the high fat diet (530±85 revertants) compared with the LF, LFb, LB diet (230±25). The RAL was found to be greatest in rats fed the high fat diet (5.44×10^{-9}), followed by the high Fb diet, high B diet and control diet (4.48, 3.85 and 3.50×10^{-9} respectively). Thus intake of a high fat human diet increased both the in vitro activation of these carcinogens and background levels of DNA adducts in vivo.

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ANTILEISHMANIAL ACTIVITIES OF SOME KNOWN AND POTENTIAL DRUGS ON LEISHMANIA DONOVANI PROMASTIGOTES

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A large number of antiprotozoal drugs and some potential compounds had been screened for antileishmanial activity against Leishmania donovani promastigotes. In a blood-free semisynthetic liquid medium minimum inhibitory concentration(MIC) of each of these drugs were determined. Amphotericin B was most effective with Pentamidine as the next to it. Stibogluconate was most ineffective. Primaquine phosphate and halogenated 8-hydroxyquinolines were also highly effective. Amongst the new compounds PALA(N-Phosphon-Acetyl-L-Aspertic acid) was found to be fairly active and a bis-naphthaquinone Diospyrin and an anticancer antibiotic Jawaharene fraction JF1 were found to be remarkably potent. Inhibition of respiration of the organism by all kinds of drugs and compounds had been found to be positive excepting a few.

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DOES ATIPAMEZOLE AFFECT THE HEPATIC ELIMINATION OF MEDETOMIDINE IN BEAGLE DOGS?

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Medetomidine (M) is an α_2-adrenoceptor agonist used as an analgesic sedative for dogs and cats. Atipamezole is an α_2-antagonist which can be used to revert te effects of M. Both drugs are eliminated by biotransformation. In order to find out possible pharmacokinetic interactions, single i.m. doses of M and A were administered separately to 6 Beagles. Then a dose of M, followed 30 min later by A, was given. Drug concentrations were determined by GC-MS and pharmacokinetic parameters calculated by PCNONLIN. Statistical analysis of the results (ANOVA) revealed significant differences in the kinetic parameters of medetomidine between treatments Atipamezole decreased the AUC of M from 41.3 ng h ml^{-1} to 28.6 ng h ml^{-1} ($p<0.005$), $t_{\frac{1}{2}}$ from 1.44 h to 0.87 h ($p<0.05$) and increased CL from 21 ml min^{-1} kg^{-1} to 31 ml min^{-1} kg^{-1} ($p<0.05$). Differences in $t_{\frac{1}{2}a}$, t_{max}, or C_{max} did not reach statistical significance. Total excretion (M+metabolites) in the urine or faeces was not affected. Increased elimination of M cannot be explained by interactions at the enzyme level, because the drugs have been found to inhibit each others hydroxylation by rat hepatic microsomal monooxygenases in vitro. Instead, because high doses of M can cause some hemodynamic changes, it is suggested that A by restoring the hepatic blood flow could affect the availability of M to metabolising enzymes and, consequently, increase its rate of elimination in the liver.

GLUTATHIONE (GSH) AND THE FROG SPINAL CORD ELECTRICAL ACTIVITY: INTERACTIONS AND PROTECTIVE EFFECTS.

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GSH participates in a large number of cellular mechanisms (Meister, 1981, 1983; Meister and Anderson, 1983) and is believed to protect proteins and cell membranes against peroxides and free radicals. The conjugation of this thiol with drugs could also produce a detoxication mechanism occurring in cells (Habig et al.,1983). Our preliminary results, from a study in progress, concern the direct action of GSH on spinal nerve cell electrical activity and the mechanisms underlying the protective ability of thiols against agents altering the regular cell functionality. GSH in the Ringer's perfusate, at concentrations ranging between $5x10^{-4}M$ and $10^{-2}M$, drastically and reversibly depressed spontaneous potentials from dorsal and ventral spinal roots of the 8th or 9th segment. In addition, clear depressive effects could be recorded in the evoked reflex activities. On the contrary, the disulphide (GSSG) did not affect any of the electrical spinal potential. Pretreating the cord with GSH, and then washing it away, prevented the irrevesible effect of NEM (n-ethyl maleimide) on spinal nerve cells (De Santis A. et al.,1990).
Our preliminary results show a primary direct action of GSH on the neuronal membrane of frog spinal cells. This affects the spontaneous and evoked potentials that originate from synaptic activities in a reversible manner. Assuming that GSH binds to membrane protein sulphydryl groups, as hypothesised for methanethiol (De Santis et Al., 1990), this configuration might screen the -SH groups thus preventing the oxidizing action of NEM.

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CHARACTERIZATION OF PIG LUNG CARBONYL REDUCTASE

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Carbonyl reductases with different properties have been purified from several mammalian tissues. We found that pig lung contained large amounts of pyrazole-sensitive carbonyl reductase activity, and now report the purification and characterization of the pig lung carbonyl reductase. The enzyme purified from the tissue was a tetramer with a Mr 103,000 which composed of apparent identical subunits of Mr 24,000. The enzyme reduced various aliphatic and aromatic carbonyl compounds including 3-ketosteroids with NADPH as a preferable cofactor to NADH, oxidized secondary alcohols, and catalyzed the aldehyde dismutation. Immunohistochemical study with antibodies against the purified enzyme revealed the specific localization of the enzyme in the bronchial ciliated cells, bronchiolar Clara cells, Type II alveolar cells and in the epithelial cells of the ducts of bronchial glands. These properties of the pig lung enzyme are similar to those of the enzymes found in guinea-pig and mouse lung, but the pig enzyme showed following unique properties. (1) 3α- and 3β-Hydroxysteroids were identified as the reduced products of 5β- and 5α-androstanes respectively, but the 4-pro-S hydrogen of NADPH was transferred to the carbonyl carbon atom of the ketosteroids. (2) At pH 7.0-7.5, the enzyme exhibited negative homotoropic cooperativity with respect to carbonyl substrates but not to NADPH. A lowering of pH for reaction activated the enzyme by abolishing the cooperativity. (3) Similar allosteric activation was also observed by the addition of fatty acids, of which cis-unsaturated fatty acids such as oleic acid and arachidonic acid were most effective activators showing Ka values of 2.2-14 μM.

METABOLISM OF THE EXPERIMENTAL ANTITUMOUR AGENT N-[2-(DIMETHYLAMINO)ETHYL]-ACRIDINE-4-CARBOXAMIDE IN MOUSE HEPATOCYTES

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Acridine-carboxamide (AC) is an experimental antitumour agent with high activity against solid tumours in mice. In vivo data indicate that AC is extensively metabolised. The aim of this study was to evaluate the metabolism of AC in mouse hepatocytes.

AC metabolism in mouse hepatocytes was time and dose-dependent. After a 2h incubation with 250 μM ^{3}H[AC] the overall metabolism was >90%. From HPLC analysis with synthetic standards we have identified monomethyl-AC as the major metabolite, as well as the N-oxide and amine derivatives. The formation of these metabolites with cell homogenate was NADPH dependent. Cell mediated formation of the corresponding acridones was also detected and experiments with hepatic cytosol indicate the involvement of aldehyde oxidase in this reaction. In addition significant formation of as yet unidentified polar metabolites occurred (~45%). Incubation with β-glucuronidase indicated the presence of several glucuronides. Further studies to identify these products are in progress. Two of these conjugates as well as N-oxide-AC and monomethyl-AC have been detected to date in mouse urine and faeces.

Thus these results indicate that the hepatocyte system is a useful tool to investigate the in vivo pathways of AC metabolism.

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MOLECULAR CLONING OF THE RAT KIDNEY CYTOSOLIC CYSTEINE CONJUGATE β-LYASE GENE.

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Two λgt11 cDNA libraries, derived from Wistar and Sprague-Dawley rat kidney mRNA, were screened with a monospecific polyclonal antibody raised against electrophoretically homogeneous rat kidney cytosolic β-lyase. In total four cDNA 'inserts' were isolated corresponding to 0.3, 0.9, 1.0, and 2.9 kb of rat sequence. All four cDNA inserts led to the formation of β-galactosidase fusion proteins in E.Coli lysogens which cross-reacted with the β-lyase antibody, suggesting that cDNAs corresponding to coding regions of the β-lyase gene had been isolated. Northern blot analysis of rat liver and kidney RNA using the 0.9kb cDNA as probe indicated that it corresponded to a kidney-specific mRNA 2.9kb in size. The four cDNAs have been sequenced by the dideoxy chain termination method and are currently being compared to amino acid sequences from purified renal cytosolic β-lyase to further substantiate isolation of the β-lyase gene. The 0.9kb cDNA insert has also been used to examine the *in vivo* modulation of β-lyase mRNA levels by cysteine conjugates.

METABOLISM OF HUMAN ENDOTHELIAL GLUTATHIONE DURING OXIDANT INSULT: INVOLVEMENT OF PROTEIN-SPECIFIC S-THIOLATION

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Confluent human umbilical vein endothelial cells were treated with diamide, t-butyl hydroperoxide or glucose oxidase/glucose and changes in glutathione oxidation, protein S-thiolation and efflux of cystein equivalents were examined. Glutathione was rapidly oxidized to similar amounts in all three test systems. Diamide caused S-thiolation of a variety of proteins (with molecular masses of 34, 24 and 14 KDa), but only one protein, of molecular mass 44 KDa was extensively modified. This protein binding was reversible to some extent with longer incubation time. t-Butyl hydroperoxide caused modification of proteins with molecular masses of 24 and 14 KDa and glucose oxidase/glucose of proteins at 39, 24 and 14 KDa. Extensive efflux of cysteine equivalents from the cells was observed which was highest during diamide treatment.

This study shows that S-thiolation of proteins is an important metabolic response to oxidant insult in endothelial cells and that the specificity of the response depends on the agent used.

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FENTON-REACTIVE IRON BINDING TO ISOLATED RAT HEPATOCYTES AND ITS INVOLVEMENT IN PEROXIDE-MEDIATED TOXICITY

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Free or bound iron that is able to participate in oxidation-reduction reactions may mediate free-radical and oxidative biochemical toxicity. Iron chelators that prevent redox cycling, such as desferrioxamine and *o*-phenanthroline inhibit this toxicity. We developed a procedure to quantify subnanomolar levels of Fenton-Reactive Iron (FRI), which is that pool of redox-active iron capable of participating in a model Fenton reaction. Exogenous iron (ferrous ammonium sulfate) binds to hepatocytes and displays positive cooperativity, with apparent kinetic constants of K_d = 10.6 μM, B_{max} = 20.7 nmol/mg protein, and the Hill Coefficient = 1.4. Binding of FRI occurred within a few seconds and the amount of FRI associated with hepatocytes was stable for at least an hour, indicating the lack of time-dependent FRI internalization. Lipid peroxidation was promoted by free extracellular iron, but not by bound iron. Conversely, bound rather than free iron enhanced concentration-dependent *t*-butyl hydroperoxide-mediated toxicity, suggesting toxicological relevance only of bound iron. The divalent cations Mn^{2+} and Co^{2+} inhibited iron-mediated hepatocyte killing in the presence of *t*-butyl hydroperoxide, but only if added prior to Fe^{2+}. Mn^{2+}, but not Co^{2+}, inhibited Fe^{2+}-mediated lipid peroxidation regardless of the order of addition. The results suggest that iron remains redox-active upon binding to sites on the hepatocyte membrane. Furthermore, this bound FRI is involved in peroxide-mediated toxicity, but not in lipid peroxidation. We are attempting to identify these sites and their role in iron homeostasis or other types of chemical intoxication. (USPHS grant ES-03373 and the Swedish Medical Research Council)

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BOTH CHEMOPROTECTION AND CHEMICAL TOXICITY MAY BE MEDIATED BY CHANGES IN MEMBRANE FLUIDITY

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Low concentrations of the antioxidant butylated hydroxytoluene (BHT) protect against the toxicity of other chemicals in isolated rat hepatocytes. However, higher concentrations exhibit intrinsic dose-dependent toxicity. In the RBC osmotic fragility assay, low concentrations of BHT also protected against osmotic fragility, while toxic BHT concentrations increased osmotic fragility. The BHT-mediated alterations in osmotic fragility correlated with changes in membrane fluidity, determined by fluorescence polarization, with increased fluidity associated with osmolysis. In rat hepatocytes high BHT concentrations also increased plasma and mitochondrial membrane permeability and fluidity. For BHT, chemoprotection at low concentrations, or intrinsic toxicity at higher concentrations, are respectively associated with decreases or increases in membrane fluidity. Menadione toxicity was mitigated by antioxidants at low but not high concentrations of menadione. Menadione produced a time-dependent and concentration-dependent inhibition of lipid peroxidation in intact hepatocytes, correlating with an increase in the antioxidation potency of ethyl acetate extracts of cells incubated with menadione. Furthermore, these extracts increased osmotic fragility in red blood cells, which correlated with an increase in membrane fluidity. Menadione also increased membrane fluidity in hepatocytes. Dithiothreitol protected hepatocytes from toxicity at low but not high concentrations of menadione. The results suggest that while menadione toxicity in hepatocytes is mediated by oxidative stress and arylation at low concentrations, toxicity at higher concentrations is the result of arylation and increased membrane fluidity. (Supported by USPHS grant ES-03373 and the Swedish Medical Research Council)

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PHYTOALEXINS : ROLE OF MICROSOMAL CYTOCHROME P450s AS PART OF THE DEFENSE MECHANISM OF BEAN SPROUTS

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Phytoalexins are low molecular weight compounds, known to function as key components of the chemical defense mechanism of many nutritional legumes against a diverse group of pests including insects, fungi and bacteria. Many phytoalexins, or intermediates in the synthesis of these compounds, are recognized as natural toxic chemicals frequently present in the diet of humans. The biosynthesis of isoflavonoid phytoalexins proceeds via a multi-step pathway which begins with phenylalanine. In higher plants a number of different cytochrome P450 monooxygenases participate in the biosynthetic pathways leading to phytoalexins, flavonoids, lignins, chalcones, hormones etc. Microsomal monooxygenase reactions are dependent not only on different cytochrome P450s, but also on the function of a common NADPH-cytochrome P450(c) reductase(EC.1.6.2.4), a flavoprotein, that transfers electrons necessary for the activation of oxygen at the heme center of a cytochrome P450.

To understand the mechanisms of function of P450s for phytoalexin synthesis in plant systems, we have solubilized and purified to electrophoretic homogeneity the NADPH-cytochrome P450(c) reductase from the microsomal fraction of 4 day old mung bean sprouts. The purified mung bean reductase showed a single protein band on SDS-PAGE with a molecular weight of 82,000 daltons. One of the cytochrome P450 dependent enzymes in the biosynthetic pathway of phytoalexins, cinnamic acid 4-hydroxylase, was assayed using microsomes from different ages of etiolated and wounded mung bean sprouts. The inhibitory effects of anti-mung bean reductase on the microsomal NADPH-cytochrome P450(c) reductase and cinnamic acid 4-hydroxylase was studied. The participation of plant NADPH-cytochrome P450(c) reductase in different cytochrome P450 dependent monooxygenase reactions, is currently being investigated.

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IN VITRO METABOLISM OF A NOVEL ORGANOPHOSPHORUS INSECTICIDE : ACTIVATION BY RAT AND JAPANESE QUAIL HEPATIC MICROSOMES

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Metabolism is one of the important determinants of the toxicity of a xenobiotic. Phosphorothionates may be detoxified and/or activated during biotransformation mediated by microsomal monooxygenases. The activation of such a compound, a thiol analogue of monocrotophos synthesized at IICT-Hyderabad, India was studied using hepatic microsomal preparations of rat and Japanese quail. The NADPH-dependent inhibition of hepatic microsomal B-esterases (α-naphthyl acetate esterase) provided a measure of its activation to an oxon by oxidative desulphuration. Interestingly, the inhibition of α-NaE activity by the analogue was much faster in the quail than in the rat during the first 3 minutes of the study. This difference may be due to the known deficiency of 'A'-esterases in the bird in contrast to the rat where high 'A'-esterase activity may lead to rapid hydrolysis of oxon that is formed. Also a Warfarin-resistant strain of rats showed less inhibition of hepatic microsomal α-NaE than a susceptible one under similar experimental conditions. Thus a resistant strain of rats appears to activate the thiol analogue more slowly than the susceptible one does and/or hydrolyse the activated product faster. *In vitro* metabolism of the [^{14}-C] monocrotophos analogue by rat hepatic 11~S and 105~S fractions was also studied. The analyses of metabolic extracts by radio TLC Scanner indicated that in addition to oxidation by microsomal monooxygenases the analogue is detoxified non oxidatively, probably by the hydrolysis of the ester bond. The study throws some light on species and strain differences in metabolism which may be expected to lead to corresponding differences in toxicity. In the context of ecotoxicology this approach can provide useful guidelines in regard to risk assessment for pesticides.

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GLUTATHIONE CONJUGATION OF BROMOSULFOPHTHALEIN (BSP) IN PERFUSED RAT LIVER

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Bromosulfophthalein (BSP) is a highly protein bound hydrophilic compound used for studies of hepatic transport and glutathione conjugation. The fate of BSP was studied in the perfused rat liver preparation wherein the unbound fraction of BSP in plasma perfusate (20% red cell and 1% albumin) was extremely low (< 1%). Both BSP and its glutathione conjugate (BSP-GSH) were quantified by a newly developed solvent-gradient HPLC method. A range of concentrations (0.5 to 2000 µM) was used; BSP was delivered into the rat liver via the portal vein (10 ml/min) for 50 to 60 min. The maximum steady state of BSP extraction ratio (0.45) was found to exist at input concentration < 2 µM, and this declined gradually with increasing BSP concentrations.
BSP-GSH was the only metabolite found, appearing only in bile and not hepatic venous perfusate. BSP was excreted into bile, but at much lesser rates in comparison to those for BSP-GSH; the excretion of BSP-GSH and BSP into bile accounted completely for the loss of BSP in perfusate. BSP-GSH biliary excretion rates began to display saturation patterns at BSP concentrations > 200 µM, whereas depletion of hepatic GSH was observed at BSP concentrations ≅ 100 µM. The data suggest that GSH plays a rate-limiting role in the formation of BSP-GSH at high BSP concentrations.

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PRECLINICAL PHARMACOKINETICS OF STOBADIN

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Stobadin, cis-(-)-2,3,4,4a,5,9b-hexahydro-2,8-dimethyl-1H-pyrido-[4,3-b]indole, is a gamma-carboline derivative with relevant *in vivo* antioxidant properties [1]. It is an active stereoisomer of racemic carbidine, which is used as a neuroleptic agent. So far N-oxide and N-monodesmethyl metabolites of stobadin were evidenced *in vitro* and *in vivo*.

^{3}H-labelled stobadin was synthesised for experimental pharmacokinetic studies [2]. A TLC method was developed for the determination of ^{3}H-stobadin in plasma of experimental animals [3]. For studying the bioavailability of orally administered stobadin in dogs an HPLC method was established [4].

The basic pharmacokinetic parameters of stobadin dihydrochloride found in rats after iv administration are: $t_{1/2\ el}$ = 85.6 min, V_{ss} = 4.8 $l.kg^{-1}$, Cl_{tot} = 105 $ml.min^{-1}.kg^{-1}$ [5]. Pharmacokinetic parameters valid for dogs after drug po administration are: $t_{1/2}$ = 312.2 min, Cl_{tot} = 6.99 $ml.min^{-1}$ [4].

1. Beneš L., Štolc S. Drugs Future 14 (1989) 135.
2. Marko V., Filip J., Uhrín D., Trnovec T., Beneš L. J. Label. Comp. Radiopharm. 27 (1988) 35.
3. Bittererová J., Šoltés L., Kállay Z., Trnovec T. Pharmazie 45 (1990) 437.
4. Šoltés L., Kállay Z., Bezek Š., Fedelešová V. Biopharm. Drug Dispos. 12 (1991) 29.
5. Kállay Z., Bittererová J., Brejcha A., Faberová V., Bezek Š., Trnovec T. Arzeim.-Forsch./Drug Res. 40 (1990) 974.

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INDUCTION AND INHIBITION OF HEPATIC DRUG-METABOLIZING ENZYMES IN THE RAT BY ALBENDAZOLE, A BENZIMIDAZOLE ANTHELMINTIC.

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Male Sprague-Dawley rats were treated with various doses of albendazole for 10 d. The treatment raised total cytochrome P-450 by factors of 1.3, 1.5, and 2 in the hepatic microsomes of rats given 10, 20, and 50 mg/kg, respectively. When doses of 100 mg/kg were given,the cytochrome P-450 concentrations were similar to those in the microsomes of control ? ((untreated) rats. For doses between 2 and 50 mg/kg, we found simultaneous dose-dependent increases of the activities of 7-ethoxyresorufine O-deethylase, p-nitroanisole O-demethylase, and type-1 UDP-glucuronosyltransferase (UDPGT). At doses of 100 mg/kg, the increases of the 7-ethoxyresorufine O-deethylase and type-1 UDPGT activities were less and the p. nitroanisole 0-demethylase activity was not increased at all. Electrotransfer and immunological staining (immunoblotting) showed that cytochromes P-450c and were induced ; the levels of both these isoenzymes were lower at 100 than at 50 mg/kg.

Polyclonal antibodies directed against cytochrome P-450 did not reveal any significant change in this isoenzyme, whereas the levels of two isoenzymes immunologically related to it fell sharply at 100 mg/kg. At 20 mg/kg and above, the aminopyrine and ethylmorphine N-demethylase activities were inhibited, while the activities of erythromycin and benzphetamine N-demethylase and of aniline hydroxylase were inhibited only with treatment at 100 mg/kg. This inhibition at a low administered dose seems to be due to a metabolite of albendazole.

H. Souhaili El Amri, X. Fargetton, P. Delatour and A.M. Batt.
Xenobiotica, 17, 1159-1168, 1987.

PERCUTANEOUS ABSORPTION OF VOLATILE CHEMICALS IN THE RAT

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The percutaneous absorption of chemicals is often first estimated in the rat. A radiolabelled compound is applied dermally under a non-occlusive dressing and the radioactivity which is subsequently eliminated or retained is determined. There are practical difficulties in studying the percutaneous absorption of volatile compounds and the studies described here were designed to address these problems.

A variety of saddles, chambers, and vessels has been used to study percutaneous absorption in vivo. The function of these devices is (a) to circumscribe a defined area of application, (b) to prevent exfoliated skin from contaminating collected urine and faeces, and (c) to exclude other routes of absorption that may result from grooming. However, where a chemical is highly volatile, discrimination between exhaled material and that volatilising from the application site is not possible using existing methodology.

At HUK we have modified the basic design used for non-occlusive application to offer an additional function ie to trap highly volatile test materials released from the application site, thus discriminating between exhaled material and that volatilising from the application site. The device consists of a glass saddle in which an appropriate adsorbent material is contained between two glass sinters held above the application site. The saddle plus contents is firmly fixed to the application site using a cyanoacrylate adhesive.

Prior to performing a study, consideration must be given to ensure (a) that the adsorbent material is appropriate for its intended purpose, (b) that an air-tight seal is present between saddle and application site for the duration of the study, and (c) that adsorbed material can, if required, be desorbed from the adsorbent material thus enabling quantification and identification of volatilised material.

Studies conducted in this laboratory have provided data which suggest that the above saddle is highly efficient for its intended purpose for up to 72 h post-attachment when used with a diverse range of chemicals and formulations including liquids, granules and powders.

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AN ENZYME MECHANISM STUDY AND STRUCTURE ACTIVITY RELATIONSHIPS FOR RAT RENAL CYTOSOLIC ß-LYASE.

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Cysteine conjugate ß-lyase, a pyridoxalphosphate (PLP) dependent enzyme, catalyses the C-S cleavage of cysteine conjugates of a number of xenobiotics to yield pyruvate, ammonia and sulphur containing metabolites. Reactive sulphur containing metabolites are held responsible for the nephrotoxicity of some halogenated alkanes and alkenes. As yet little is known about structure activity relationships for cysteine conjugate ß-lyase enzyme. Therefore studies are needed to get more insight into the mechanisms by which this enzyme catalyses the ß-elimination reaction. This reaction can be divided into a number of steps which, in principle, can all be rate limiting; 1) Substrate bindig and formation of an aldimine of PLP and the nitrogen of the cysteine moiety of the substrate. 2) α-proton abstraction by a protein (active site) base. 3) elimination of the thiol from the activated enzyme-substrate complex. 4) The hydrolysis of the ketimine complex to PLP, ammonia and pyruvic acid.

In this study the following structurally related L-cysteine conjugates were used; 1,1,2,2-tetrafluoroethylene-L-cysteine, 1-chloro-1,2,2-trifluoroethylene-L-cysteine, 1,1-dichloro-2,2-difluoroethylene-L-cysteine and 1,1-dibromo-2,2-difluoroethylene-L-cysteine. For these substrates the activation energies (E_{act})were determined by means of Arrhenius plots obtained from in vitro incubations with rat renal cytosolic fraction. These activation energies were used to quantify difference in activity between the four substrates. To answer the question whether the observed differences in activation energy between the four substrates could be explained by a difference in substrate binding, i.e. the formation of the internal aldimine, competitive inhibition experiments were carried out using amino-oxyacetic acid (AOA) as an inhibitor. With the resulting Dixon plots it could be demonstrated that in the case of the four cysteine conjugates tested there was no difference between the K_i values for AOA. For these cysteine conjugates it is concluded that the formation of the internal aldimine is not rate limiting.

In order to determine whether α-proton abstraction is the rate limiting step, the corresponding α-deuterium analogue of 1-chloro-1,2,2-trifluoroethylene-L-cysteine was synthesized. This compound was tested for ß-elimination activity in both the rat renal cytosol and a chemical ß-lyase mimetic system. From the observed isotope effect k_{H/k_D}=4 it is concluded that this step is most likely rate limiting in the ß-elimantion of this conjugate. This conclusion was supported by the fact that the activation energy for the ß-elimination of this conjugate was increased by 40%. This work is now being expanded to the other three L-cysteine conjugates mentioned.

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INVESTIGATION ON A POSSIBLE INDUCTION OF AZT GLUCURONIDATION BY RIFABUTIN AND RIFAMPICIN IN HUMANS

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Zidovudine (AZT) can increase the life expectancy of patients with the acquired immunodeficiency syndrome (AIDS). 5'-glucuronyl-AZT (GAZT) is the only known metabolite of AZT in man. Plasma levels of GAZT are about 6-7-fold higher than those with AZT. GAZT has no anti-HIV activity. The aim of this study was to assess the influence of multiple doses of rifabutin (RIF), a new rifamycin derivative, and of rifampicin (RIP) on the pharmacokinetics and metabolism of AZT. RIF and RIP induce cytochrome P-450 enzymes, but it is not known if they induce also glucuronyl transferase enzymes. Two groups of ten healthy volunteers were treated with AZT (200 mg, p.o.) on day 1, then with RIF (300 mg/day, p.o.) or with RIP (600 mg/day, p.o.) from day 2 to day 11. On day 12 they received again 200 mg AZT. Plasma levels of AZT and of GAZT were assayed by HPLC. Urinary excretion could be studied only for AZT. C_{max} (1285 ng/ml, mean values) and $AUC_{0-\infty}$ (1395 ng.h/ml) of AZT were significantly decreased by RIF treatment (803 ng/ml, 953 ng.h/ml). C_{max} (1345 ng/ml) and $AUC_{0-\infty}$ (1220 ng.h/ml) of AZT were also significantly lowered by RIP treatment (476 ng/ml, 586 ng.h/ml). T_{max} and $t_{1/2}$ of AZT were not changed by both antibiotics. 11% of the dose was recovered in the urine as AZT and this value was not modified by RIF, but significantly decreased by RIP. No significant difference was observed for the C_{max}, T_{max}, $AUC_{0-\infty}$ and $t_{1/2}$ of GAZT following treatment with RIF and RIP. In conclusion RIF and RIP appear to induce AZT metabolism in man, although the metabolic pathway enhanced remains to be investigated.

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INVOLVEMENT OF CELLULAR SIGNAL TRANSDUCTION IN DRUG-EVOKED PEROXISOMAL ENZYME INDUCTION IN RAT LIVER

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We have shown for the first time that calcium antagonist such as nicardipine, nifedipine and diltiazem suppressed the clofibrate-evoked induction of the activities of peroxisomal enzymes. For elucidating the involvement of signal transduction system in the process of drug-evoked peroxisomal enzyme induction, in the present study we examined the effect of trifluoperazine and H-7 on the induction of peroxisomal fatty acyl-CoA oxidizing system(FAOS) and carnitine acetyltransferase(CAT) by clofibrate.

When trifluoperazine was co-administered with clofibrate to rats for 1 week, the clofibrate-evoked increase in the activities of FAOS and CAT was markedly suppressed. This suppressive effect was found in in vitro experiment using isolated hepatocytes. Similar suppressive effect was found when H-7, a protein kinase C inhibitor, was co-administered with clofibrate, bezafibrate or Wy-14,643. Thus, the suppressive effect of the inhibitors was found not to depend on the structure of peroxisome proliferators. Furthermore, the increase in the activity of microsomal omega-oxidation known to be co-induced with peroxisomal enzymes by peroxisome proliferators, was also suppressed by calcium antagonists. On the other hand, the induction of microsomal enzymes by phenobarbital was not suppressed by calcium antagonists.

These results show that complicated cellular signal transduction system including calcium-, calmoduline-and PKC-related process would be involved in the drug-evoked peroxisomal enzyme induction.

1) H. Itoga, H. Tamura, T. Watanabe and T. Suga: Biochim. Biophys. Acta, 1051, 21-28 (1990)

PHARMACIKINETICS OF CHINOIN-175 AND ITS METABOLITES IN DOGS

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CHINOIN-175 (Fludepril) is a new selective MAO-B inhibitor, which also inhibits the uptake of dopamine.
Using ^{14}C labelled preparation, the absorption, distribution and elimination were studied in Beagle dogs at the simple oral dose of 10 mg/kg. The quick absorption of substance was indicated by the peak concentration appeared 1 h after dosage, and an enterohepatic circulation was supposed on the basis of the secondary peak between 3 and 6 h. The main part of radioactivity was excreted in urine.
Beside the unchanged compound, its three N-dealkylated metabolites (p-F-methamphetamine, p-F-demethyldeprenyl and p-F-amphetamine) were identified in plasma and urine samples using g.l.c. - mass spectrometry (Multiple Ion Detection method).
The pharmacokinetics of the CHINOIN-175 and its metabolites were studied in Beagle dogs after single and multiple oral administration as well as single intravenous injection of non-radioactive substance at the same doses. The plasma levels were measured by g.l.c. after a derivatization procedure. The plasma concentrations of the unchanged compound were the highest in all cases, despite they decreased after the repeated dosage. Among the metabolites the peak concentration of p-F-methamphetamine was the highest, but the AUC values of p-F-amphetamine were the greatest. The absolute bioavailability of CHINOIN-175 was calculated for 78 %.

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Modulation of cinnamaldehyde depletion of reduced glutathione (GSH) and cytotoxicity in rat hepatocytes.

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trans-Cinnamaldehyde (CA) is used extensively in both the perfume and flavour industries. CA depletes GSH dose-dependently in suspended rat hepatocytes (1). We have modulated the metabolism of CA and studied the effect of this on CA's depletion of GSH and cytotoxicity.

Hepatocytes from male F344 rats were suspended at 10^6 viable cells/ml and treated with test compound or DMSO. Cells were incubated at 37°C under 95% O_2/5% CO_2 and samples were taken at intervals up to 6 hours. Cell viability was determined by lactate dehydrogenase (LDH) leakage into the medium.

Addition of 0.2% ethanol immediately prior to CA treatment increased the GSH depletion by $5x10^{-4}$M and 10^{-3}M CA over 6 hours, apparent only after 1 hour (see table). Ethanol had no significant effect on CA cytotoxicity.

GSH levels as a % of control after 6 hours incubation (n=3)

concentration(M)	CA only	CA + 0.2% ethanol
$5x10^{-4}$	103.9 ± 7.6	50.8 ± 13.3
10^{-3}	61.9 ± 7.7	38.4 ± 12.1

The aldehyde dehydrogenase inhibitor, cyanamide (10^{-3}M), enhanced the depletion of GSH by $5x10^{-4}$M CA (% control GSH ± se: CA only = 87.3 ± 2, CA + cyanamide = 42.3 ± 2.4) without increasing its cytotoxicity. Cyanamide caused no further depletion by 10^{-3}M CA, but the cytotoxicity of 10^{-3}M CA was significantly increased by cyanamide (% total LDH leakage after 6 hours ± se; control = 40.5 ± 2.3, 10^{-3}M CA = 55.5 ± 10.5; 10^{-3}M CA + cyanamide = 90.9 ± 6.7).

10^{-3}M cyanamide and 0.2% ethanol together did not increase GSH depletion or % LDH leakage by CA above that resulting from either pretreatment alone.

We conclude that the redox status of the hepatocyte is altered when ethanol is introduced prior to CA treatment, resulting in a prolonged lifespan of the aldehyde and a more profound GSH depletion. Aldehyde dehydrogenase inhibition allows greater GSH conjugation and causes CA to exceed the cytotoxic threshold of the hepatocyte.

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(1) Swales, N.J. and Caldwell, J. J.Hum. Exp. Tox. in press.

METABOLISM OF BUNAZOSIN IN RATS AND HUMANS

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Bunazosin hydrochloride (E-643) is a potent and selective post-synaptic α_1-adrenoceptor blocking agent. In order to clarify the metabolic fate of E-643 in humans, the human urine and feces after oral administration of ^{14}C-labeled E-643 were analysed. Identification of the metabolites of E-643 was performed by FAB-Mass and NMR. The results of qualitative analyses of the metabolites were compared between rats and humans.

The following five metabolic pathways were recognized; 1: N-glucuronidation of E-643, 2: 7-demethylation and its N or O-glucuronidation, 3: 6-demethylation and its N or O-glucuronidation, 4: α or β-hydroxylation of E-643 and its O-glucuronidation, 5: hydroxylation of the homopiperazine ring of E-643 and the cleavage of the ring. The main metabolic pathways of E-643 were routes 2 and 5 in rats and routes 1, 3 and 5 in humans. The percentages of the metabolites of E-643 to the dose differed between rats and humans, however the metabolic pathways of E-643 were thought to be similar.

These results suggest that the metabolic pathways of E-643 differ from those of prazosin and doxazosin, which are E-643 analogues with the same quinazoline structure.

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RENAL HANDLING OF A NEW THROMBOXANE SYNTHETASE INHIBITOR (DP-1904) IN THE RAT.

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DP-1904, [6-(1-imidazolymethyl)-5,6,7,8-tetrahydronaphthalene-2-carboxylic acid hydrochloride hemihydrate] is a new potent and long-acting thromboxane synthetase inhibitor. Preclinical and Phase I studies have shown in animals and humans that renal excretion is a major route of elimination. The specific processes involved in the excretion of such an amphoteric drug are, however, unknown. Accordingly, the renal excretory characteristics of DP-1904 were studied in anesthetized rats. After rapid intravenous administration of a 10 mg/kg dose, the ratio of renal clearance (CL_R) of DP-1904 to glomerular filtration rate (GFR) was 1.40 ± 0.09 (mean ± SE). Since DP-1904 plasma binding was greater than 90%, this result indicated extensive secretion into urine. During steady-state infusion, CL_R was markedly reduced from 6.17 ± 0.63 in the control period to 2.41 ± 0.58 ml/min/kg when probenecid was coadministered and the CL_R/GFR ratio fell from 1.63 ± 0.12 to 0.66 ± 0.06 ($p < 0.001$). More modest (30%) but significant ($p < 0.05$) decreases were observed following coadministration of quinine. Thus, the urinary excretion of DP-1904 involves both filtration and active secretion, with the latter primarily reflecting transport by the anion carrier system of the proximal tubule.

TIME DEPENDENT CONCEPTAL METABOLISM IN VITRO OF QA 208-199 AFTER PREINDUCTION IN UTERO WITH VARIOUS CYTOCHROME P-450 ENZYME INDUCERS.

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The rat conceptus metabolizes the lipoxygenase inhibitor N-hydroxy-N-methyl-7-propoxy-2-naphtalinethanamine (QA 208-199, QAB) in vitro. In addition, preinduction in utero with the cytochrome P-450 enzyme inducers Aroclor 1254^R (AC), 3-methylcholanthrene (3-MC) or phenobarbital (PB) revealed that pretreatment with PB led, after 48 h of culture, to a statistically significantly increased impairment of embryonic differentiation in 9.5 day old conceptuses in vitro. This indicated that conceptal cytochrome P-450 species are involved in the QAB metabolism in vitro. In this study, pregnant dams were given ip injections of either AC, 3-MC or PB prior to the dissection and 2 day culture of 10.5 day old embryos. Embryotoxic effects were evaluated in the conceptuses and metabolites were determined in the culture media and conceptal tissues with HPLC. In non-preinduced 10.5 day old embryos QAB caused less embryotoxicity compared to 9.5 day old ones. In preinduced conceptuses of 10.5 days yolk sac vascularization was less affected and less embryonic anomalies were found compared to conceptuses cultured from 9.5-11.5 days. AC and PB pretreatment caused a decreased morphological score without changes in overall growth. Analysis of QAB and its metabolites in culture media and conceptal tissues at 12.5 days showed different levels of metabolites depending on the used inducer. We conclude that 10.5 day old embryos were less susceptible to QAB embryotoxicity in vitro as compared to 9.5 day old ones after preexposure in utero to PB. In addition, the response to preinduction seemed to be age dependent.

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EFFECT OF THE FLUORO SUBSTITUENTS ON THE REGIO- AND STEREOSELECTIVITY OF CYTOCHROME(S) P450-CATALYZED OXIDATION OF POLYCYCLIC AROMATIC HYDROCARBONS

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Substitution by fluorine at an aromatic double bond usually results in a complete inhibition of cytochrome P450-mediated oxidative metabolism at the substituted double bond. In many cases, oxidative metabolism is also inhibited at the double bond peri to the substituted double bond. Our studies show that while 6-fluorobenzo[c]phenanthrene (6-fluoro BcPh) with a K-region fluoro substituent is not oxidized at the substituted double bond by rat liver cytochromes P450, the fluoro-substituted double bond in 6,7-difluoro BcPh (a fluoro substituent in each of the two K-regions) is metabolized by these enzymes to form a K-region fluoro quinone as the predominant metabolite. The predominant K-region metabolites formed by rat liver microsomes from BcPh and 6-fluoro BcPh are the trans dihydrodiol and the arene oxide, respectively. This implies that the peri fluoro substituent inhibits the epoxide hydrolase-catalyzed hydrolysis of the arene oxide. Our evidence based on CD spectroscopy indicates that the 6-fluoro substituent also causes a reversal of the stereoselectivity of the cytochromes P450 in liver microsomes from 3-methylcholanthrene-treated rats such that the predominant enantiomers of the K-region arene oxides formed from BcPh and 6-fluoro BcPh have opposite absolute configuration. This is the first example in which a fluoro substituent reversed the stereoselectivity of cytochrome P450 in the formation of an arene oxide.

HYDROXYLATION OF LAURIC ACID AND TESTOSTERONE BY RAT LIVER CYTOCHROME P450IVA PROTEINS IN RECONSTITUTED SYSTEMS

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HYDROXYLATION OF LAURIC ACID BY PURIFIED RAT LIVER P450IVA1 AND A MIXTURE OF P450IVA2 AND P450IVA3 WAS INVESTIGATED IN A RECONSTITUTED SYSTEM. 12-HYDROXY LAURIC ACID WAS THE ONLY METABOLITE DETECTABLE WITH P450IVA1. ITS RATE OF FORMATION, 12.24 NMOL/(MIN X NMOL P450), WAS REDUCED TO 25% BY EQUIMOLAR AMOUNTS OF CLO5, A MONOCLONAL ANTIBODY (MAB) WITH HIGH SELECTIVITY FOR ALL THREE P450IVA PROTEINS. IN CONTRAST, AN ABOUT EQUIMOLAR MIXTURE OF P450IVA2/A3 CATALYZED BOTH, THE FORMATION OF 11- AND 12-HYDROXY LAURIC ACID AT A RATIO OF 1:3 YIELDING A TOTAL OF 11.8 NMOL/(MIN X NMOL P450). 25μG (166 PMOL IGG) OF THE P450IVA2/A3-DIAGNOSTIC MABS CLO5 AND CLO6 REDUCED LAURIC ACID 11- AND 12-HYDROXYLATION OF 150 PMOL P450IVA2/A3 TO 76 AND 75, AND 57 AND 66%, RESPECTIVELY, WHILE BE1, A MONOCLONAL IGG DIRECTED AGAINST RAT P450IIB1/B2 SLIGHTLY INCREASED THE CORRESPONDING ACTIVITIES TO 110 AND 122% OF CONTROL. USING TESTOSTERONE AS SUBSTRATE IN THE SAME SYSTEM, P450IVA1 DID NOT EXPRESS ANY CATALYTIC ACTIVITY, WHEREAS P450IVA2/A3 PRODUCED A METABOLITE COMIGRATING WITH 7α-HYDROXY-TESTOSTERONE AT A RATE OF 110±42 PMOL/(MIN X NMOL P450). THIS ACTIVITY WAS ABOLISHED BY EQUALLY MOLAR AMOUNTS OF CLO6. INHIBITION OF TESTOSTERONE 7α-HYDROXYLASE BY CLO6 WAS ALSO INVESTIGATED IN MICROSOMAL FRACTIONS FROM MALE AND FEMALE RAT LIVER AND KIDNEY. THESE FINDINGS WILL BE DISCUSSED IN THE LIGHT OF SEX-SPECIFIC EXPRESSION OF P450IVA PROTEINS AND THEIR INDUCIBILITY BY PEROXISOME PROLIFERATORS.

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IDENTIFICATION OF THE CYT-P450 ISOENZYMES RESPONSIBLE FOR THE METABOLISM OF ZOLPIDEM

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Zolpidem, a sleep inducer, is cleared mainly if not solely by metabolism and as such the interindividual variability of its pharmacokinetic parameters reflects the variability observed in a normal human population. The methyl groups are the primary sites of zolpidem metabolism with the benzylic one being the most vulnerable; this benzylic alcohol is further oxidized to a carboxylic acid which accounts for 70, 85, and 79 % of urinary metabolites in the rat, mouse and human, respectively. Our aim was to identify the cytP450s responsible for the formation of the main metabolites. The effect of various inducers was investigated with primary cultures of human hepatocytes and human liver microsomes. With the latter *in vitro* system, inhibition with competitive substrates and antibodies directed against various forms of cytP450s was also carried out. These experiments suggest that several cytP450s are involved in the metabolism of zolpidem. CytP450 IA and IIIA are responsible at least in part for the formation of the main metabolite, while the polymorphic cytP450IID6 is not involved.

THIOL MEDIATED NEPHROTOXICITY BY 1,2-DICHLOROPROPANE

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Mercapturic acid pathway may deviate to form toxic thiols via β-lyase activation. This occurrence was demonstrated for haloalkenes as chloro and fluoroethylenes. Some haloalkanes (1,2-dibromo and 1,2-dichloroethane, but not 1,2-dichloropropane) toxicity is via episulfonium ion intermediates. The aim of the present research was to study in vitro the mechanisms of 1,2-dichloropropane (DCP) nephrotoxicity using renal cortical slice model. Previous experiments showed that the parent compound decreases kidney reduced glutahione content and p-aminohippurate (PAH) accumulation. A slight lipid peroxidation was observed. In the experimental design, slices were preincubated 30 min at 37°C in a shaker bath with or without αketobutyrate (KBT) sodium salt $5x10^{-3}$ M or aminooxyacetic acid (AOAA) 10^{-3} M, specific activator and inhibitor, respectively, of β-lyase activity. The slices were then incubated 90 min at 37°C in a shaker bath with or without DCP $25x10^{-3}$ M. The incubation medium was tested for malondialdehyde (MDA) content as index of lipid peroxidation. Finally, the slices were reincubated with PAH to study the organic anion accumulation. DCP causes a significant increase ($p<0.001$) of MDA in the medium and a significant loss ($p<0.005$) of PAH accumulation. Pretreatment with AOAA partially reduced the loss of PAH accumulation ($p<0.025$) induced by DCP, whereas pretreatment with KBT enhanced the solvent toxicity increasing lipid peroxidation ($p<0.01$) and loss of PAH accumulation ($p<0.001$). In conclusion, the reported results show that DCP nephrotoxicity may be mediated by a toxic thiol formed during mercapturic acid metabolism.

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STEREOSELECTIVE INTERACTIONS OF BAMBUTEROL WITH HUMAN PLASMA CHOLINESTERASE.

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**Subsidiary of AB Astra*

Introduction

Bambuterol is a bis-dimethylcarbamate prodrug of the bronchodilator terbutaline. The therapeutic formulation of bambuterol is a racemate ((+-)B) of two enantiomers ((-)B and (+)B). Formation of terbutaline from bambuterol involves both oxidative and hydrolytic steps. One enzyme important for the hydrolytic biotransformation of bambuterol is plasma cholinesterase. The present study presents interactions of the separate bambuterol enantiomers with this enzyme.

Results and Discussion

The results suggest that (-)B has around 6-fold higher affinity than (+)B to the active site of plasma cholinesterase. The active site of cholinesterases consists of one esteratic and one anionic site. The anionic site presumably binds the positively charged nitrogen in choline esters and in bambuterol, and the fit to both parts of the active site is most likely a prerequisite for high affinity interactions. Thus, it seems likely that the two enantiomers of bambuterol differ in conformation and in relative location of the positive charge and ester bond in such a way that the simultaneous interaction with both components of the active site is facilitated for (-)B relative to (+)B. The metabolic consequence of the difference in affinity is a more efficient terbutaline formation from (-)B than from (+)B in plasma in vitro. This is of interest particularly since (-)terbutaline is a much more potent β_2-agonist than (+)terbutaline.

HUMAN CYTOCHROME P-450 IID6 AND P-450 REDUCTASE IN STABLE V79 CHINESE HAMSTER CELLS FOR INVESTIGATING DEBRISOQUINE-TYPE POLYMORPHIC METABOLISM.

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Chinese hamster V79 derived cell lines producing human cytochrome P-450 IID6 have been established. Full length cDNAs complementary to mRNA encoding human cytochrome P-450 IID6 and human cytochrome P-450 reductase were isolated and cloned by the polymerase chain reaction (PCR). An expression vector was constructed. A promotor/enhancer unit was taken from the cytomegalovirus. The vector 5'- and 3'-untranslated regions, containing the splice signals and introns IVS1 (intervening sequence 1) and IVS2, respectively, and the polyadenylation signal were derived from rabbit beta-globin gene sequence. A transcription stop signal was designed from human gastrin gene sequence. The beta-globin and gastrin gene segments were assembled from four 110-base pair synthetic oligonucleotides by a procedure of oligonucleotide assembly by bridge-mediated ligation and PCR. After subcloning the vector pieces into plasmid bluescript, the PCR isolated cDNAs, containing only coding region from the "ATG" start to "TAG" stop codons, were one-step blunt-end subcloned into a designed StuI site. A procedure of polyethylene glycol-mediated ligation created in vitro long-chain concatemers of P-450:P450 reductase:Neo selection gene with ratio of 10:4:1. Concatemeric DNA was introduced into V79 cells by electroporation and stable cells were selected. Successful high level enzymatic ability in cell culture was demonstrated by cytochrome P-450 IID6-dependent oxidation of dextromethorphan, a substrate specific for human P-450 IID6. These cell lines are useful for characterizing IID6 substrates and for screening new drugs for polymorphic metabolism in man.

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